

Application No. 09/757,673  
Supplementary Response  
January 30, 2006

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application.

**Listing of Claims:**

Claims 1-6. Canceled.

7 (Currently Amended). A method for expressing a transgene in a skeletal muscle cell, comprising the step of introducing into the cell a recombinant adeno-associated virus (rAAV) that has been produced using a helper virus and that comprises ~~comprising a~~ transgene operably linked to sequences which control its expression, wherein the rAAV is ~~at least~~ as free of contamination with a helper virus as is obtained by subjecting the rAAV to four rounds of cesium chloride gradient centrifugation and wherein the transgene is expressed in the cell.

8 (Previously Presented). The method according to claim 7, wherein the transgene encodes a secretable protein.

9 (Previously Presented). The method according to claim 8, wherein the protein is selected from the group consisting of apoE,  $\beta$ -interferon, insulin, erythropoietin, growth hormone, and parathyroid hormone.

10 (Previously Presented). The method according to claim 7, wherein the rAAV consists essentially of, from 5' to 3', 5' AAV inverted terminal repeats (ITRs), a heterologous promoter, the transgene, a polyadenylation sequence, and 3' AAV ITRs.

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Claim 11. Canceled.

Claims 12-17. Cancelled.

18 (Currently Amended). A method of delivering a transgene to a mammal comprising the step of:

administering intramuscularly to skeletal muscles of a mammal a composition comprising a biologically compatible carrier and a recombinant adeno-associated virus (rAAV) produced using a helper virus and purified therefrom, said rAAV comprising a transgene encoding a secretable protein operably linked to sequences which control expression thereof, wherein said rAAV is at least as free of adenoviral helper virus as is obtained by subjecting said rAAV to four rounds of cesium chloride gradient centrifugation, wherein the protein is secreted from rAAV-transduced muscle cells, and wherein the rAAV was purified from helper virus such that a cytotoxic immune response directed against rAAV-transduced cells of the mammal expressing the protein is not detected in the mammal .

19 (Previously Presented). The method according to claim 18, wherein the composition comprises about  $1 \times 10^8$  to about  $5 \times 10^{11}$  particles of the rAAV.

20 (Previously Presented). The method according to claim 18, wherein the composition comprises at least  $10^9$  particles of the rAAV.

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21 (Previously Presented). The method according to claim 18, wherein the composition comprises  $10^{12}$  to  $10^{13}$  genomes of the rAAV per milliliter carrier.

22 (Previously Presented). The method according to claim 18, further comprising the step of monitoring expression of the transgene in the mammal.

23 (Previously Presented). The method according to claim 18, wherein the level of contaminating adenoviral helper virus is the same as that obtained by subjecting said rAAV to four rounds of cesium chloride centrifugation.

Claim 24. Cancelled.

25 (Currently Amended). A method of delivering a transgene to a mammal comprising the steps of:

providing a recombinant adeno-associated virus (rAAV) produced using a helper virus and purified of helper virus such that an immune response to the helper virus generated by administration of rAAV is not detected upon administration of the rAAV, and

administering to skeletal muscle of a mammal intramuscularly a composition comprising a biologically compatible carrier and a the helper-free recombinant adeno-associated virus (rAAV) comprising a transgene encoding a secretable protein operably linked to sequences which control expression thereof.

26 (Previously Presented). The method of claim 25 wherein the secretable protein is selected from the group consisting of Factor IX, apoE,  $\beta$ -interferon, insulin, erythropoietin, growth hormone, and parathyroid hormone.

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27 (Currently Amended). -A The method according to claim 26~~7~~, wherein the rAAV is introduced in a composition that contains less than ~~that~~ 1 infectious units of wild-type AAV per  $10^9$  rAAV.

Claim 28 - 29. Cancelled.

30 (Currently Amended). A method of delivering apolipoprotein E (ApoE) to a mammal with atherosclerosis, said method comprising the step of administering to skeletal muscle of the mammal a composition comprising a recombinant adeno-associated virus (AAV) suspended in a biologically compatible carrier, said AAV having been produced using a helper virus and purified of helper virus such that an immune response to the helper virus generated by administration of rAAV is not detected upon administration of the rAAV.

wherein said recombinant AAV comprises (a) a 5' AAV inverted terminal repeat (ITR), (b) nucleic acid sequences encoding human apolipoprotein E (ApoE) operably linked to regulatory sequences which direct expression thereof and (c) a 3' AAV ITR, ~~wherein the recombinant AAV is at least as free of contamination with a helper virus as is obtained by subjecting the rAAV to four rounds of cesium chloride gradient centrifugation and wherein the transgene is expressed in the cell~~  
and wherein the ApoE in said composition is expressed in the mammal.

31 (Previously Presented). The method according to claim 30, wherein the recombinant AAV contains less than 1 infectious unit of wild-type AAV per  $10^9$  AAV.

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32 (Currently Amended). A method for expressing a transgene in a skeletal muscle cell, comprising the step of introducing intramuscularly into the cell a recombinant adeno-associated virus (rAAV) comprising a transgene operably linked to sequences which control its expression, wherein the rAAV has been produced using a helper virus and purified such that wherein the rAAV is free of contamination with immunogenic adenoviral helper and wherein the transgene is expressed in the cell in the absence of a destructive immune response to the rAAV-transduced cell.

33 (Currently Amended). A method for expressing a transgene in a skeletal muscle cell, comprising the step of introducing into the cell by injection a therapeutic dose of a recombinant adeno-associated virus (rAAV) comprising a transgene operably linked to sequences which control its expression, wherein the rAAV prepared using adenoviral helper was is purified of adenoviral helper such that transgene is expressed in the absence of destructive inflammation caused by contaminating helper adenovirus.

Claims 34 and 35. Cancelled.

36 (New). A method of delivering a transgene to a skeletal muscle of a mammal, wherein said method comprises the step of administering to the skeletal muscle intramuscularly a composition comprising rAAV produced using a helper virus and purified therefrom, said rAAV comprising a transgene operably linked to sequences which control its expression, wherein the transgene in the composition is expressed in the mammal in the absence of a cytotoxic immune response directed against rAAV-transduced cells of the mammal expressing transgene product in the mammal.

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37 (New). A method of delivering a transgene to a skeletal muscle of a mammal, wherein said method comprises the step of administering to the skeletal muscle intramuscularly a composition comprising rAAV produced using a helper virus and purified therefrom, said rAAV comprising a transgene operably linked to sequences which control its expression, wherein the administration does not give rise to serum antibody response associated with a cytotoxic immune response directed against rAAV-transduced cells.